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## Report Title

Reversible Nanoparticle Electronics

## ABSTRACT

Phage and yeast both exhibit excellent resiliency to electric field exposure at low currents. The viability of phage and yeast in high voltage fields was tested, opening the door to the exploration of these species as medium for self-repairing electronics applications. Furthermore, survival after high field exposure suggested that electro-spun phage are likely to remain viable, even after they are incorporated into fabrics. Pulsed electric fields were used to successfully and reversibly release a tightly binding peptide from an electrode surface, and subsequently to transfer the peptide to the opposing electrode where the peptide reattached. A bipolar electrode configuration was demonstrated enabling the electric field driven release of biomaterials from a nonconductive surface. Additionally the experiments with yeast on sapphire indicate that under the right conditions the counteracting peptide and yeast surface charges can be overcome, enabling the electrically stimulated release of yeast from a material surface.

## List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

### (a) Papers published in peer-reviewed journals (N/A for none)

**Number of Papers published in peer-reviewed journals:**

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**Reversible Nanoparticle Electronics**
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(4). Problem: Investigate how to apply the techniques of phage display or yeast display with the added variable of an electric potential to allow the discovery of peptides with electrically controllable surface affinities.

(5). Summary of Important Results:

## 1. Evolutionary Discovery of Electro-responsive Peptides

### 1.1 Phage Survivability in an Electric Field

Applying the methods of biological display in the discovery of electroresponsive peptides demands the survival of the biological agent, i.e phage or yeast, under the influence of the
electric field. A series of experiments were performed examining the viability of phage in a strong electric field. Phage bound to a metallic electrode readily withstood constant electric fields as high as 20kV/cm while still remaining infectious. Current, however, is detrimental to the phage; phage in aqueous solution become unstable as the field approaches the electro-hydrolysis point for water. Survivability experiments conducted on yeast in a pulsed electric field showed that the yeast are viable after being exposed to fields as large as 12.5 kV/cm.

### 1.2 Electric Field Driven Selection

Ideally, to discover a peptide sequence with electrically controllable binding properties, traditional phage or yeast screening methodologies can be applied while utilizing an electric field as a mediating parameter. A yeast display library was used to perform an electric-field driven selection for an aluminum substrate. The yeast is bound to the aluminum surface in PBS with 0.1% TWEEN 20. The substrate is then rinsed with DI $H_2O$ and affixed to the interior of a plastic cuvette with a 4 mm gap. A second (ground) electrode is affixed opposite the yeast-carrying (active) electrode. A series of one hundred ±5 kV, 10 ms pulses with 500 ms spacing is applied across the electrodes. The water and ground electrode are placed in SD media to grow off any cells removed from the working electrode during the field application. After four screening rounds, the yeast exhibit no enhanced removal from the working electrode under an applied potential. During some individual screening rounds, a measurable removal of yeast is observed; however, this behavior is not sustainable through subsequent screening rounds. This transient appearance of electro-responsive behavior may be related, not to the sequence of the displayed peptide, but rather to the number of expressed peptides on the yeast surface, which may be variant among screening rounds. For electric field controlled yeast display screening to be successful the yeast display technique needs to be improved by increasing homogeneity in the number of displayed peptides per yeast.

A similar technique was employed to discover electroresponsive bacteriophage. The standard phage display technique, as described by New England Biolabs was employed, but acid elution of phage from the material surface was replaced by field driven elution. Electric fields ranging from 1 – 12.5 kV/cm with both positive and negative biases were tested to elute phage with no noticeable increase in phage removal over zero field incubation.

### 1.3 Conclusions

Phage and yeast both exhibit excellent resiliency to electric field exposure at low currents. The viability of phage and yeast in high voltage fields opens the door to the exploration of these species as medium for self-repairing electronics applications. Furthermore, survival after high field exposure suggests that electro-spun phage are likely

to remain viable, even after they are incorporated into fabrics.

The isolation of an electroresponsive peptide sequence using either phage or yeast display techniques is complicated by the net negative charge of both the mannoprotein cell wall of Saccharomyces cerevisae and the PVIII major coat protein of the M13. Traditional phage and yeast display methodologies indicate that peptide sequences with high affinities for electrode materials such as ITO, aluminum, or gold are often highly positively charged. To release these peptides, the electrode to which they are bound must be biased positively. However, upon the application of a positive bias, the phage or yeast remain attracted to the electrode due to their inherent negative charge. Display techniques could potentially be used to discover an electroresponsive peptides if a display system with no inherent charge could be engineered. It should be possible to engineer a M13 library with a neutral PVIII, however it would need to be carefully design to insure the M13 could still properly assemble. Alternatively, a screening technique using a library of peptides could be developed to completely eliminate the need for a display medium.

## 2   Electrically Responsive Peptides
## 2.1 Peptide Sequences for Conducting Materials



A 12-mer yeast display library was used to discover peptides sequences that bind to gold, aluminum and platinum. A constrained phage display library was used to find peptide sequences that bind to ITO. The consensus sequences for all of these materials are highly positively charged, indicating that electrostatics plays an important role in mediating peptide binding to conductive materials.
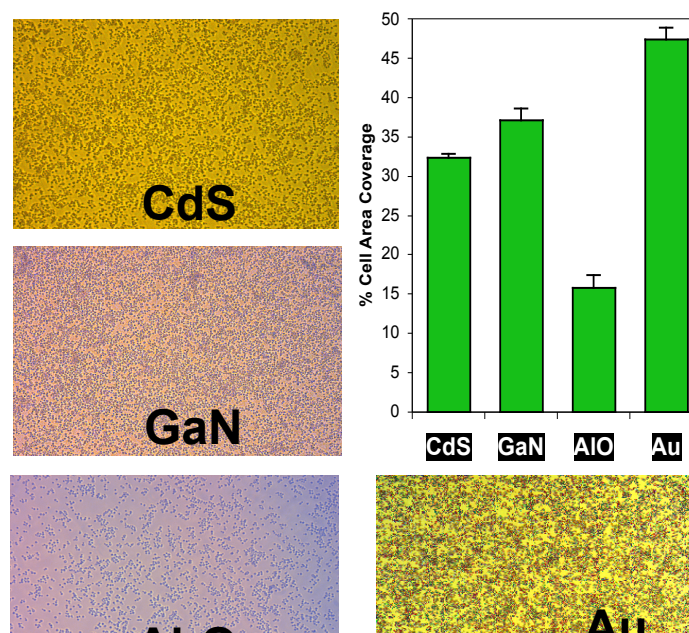
## 2.2 The AO8 Peptide

The AO8 peptide, -RSGRRRSHHHRL, was discovered from a yeast-display 12-mer random peptide library in a screen against CdS. AO8 yeast binding experiments with additional substrates (Figure1) indicate that this peptide has the ability to bind a wide range of different material surfaces, including CdS, $SiO_2$, Sapphire and potential electrode materials such as Au and Al.

A modified version of the AO8 peptide (ac-KGGGRSGRRRSHHHRL-cooh) was synthesized, altered with a tri-glycine linker to a lysine to enable fluorescent labeling of the primary amine on the lysine. The AO8 peptide exhibits less breadth of material affinities when it is no longer expressed on the yeast surface. The AO8 peptide binds primarily to oxide type materials such as aluminum and $SiO_2$, and to insulating materials.
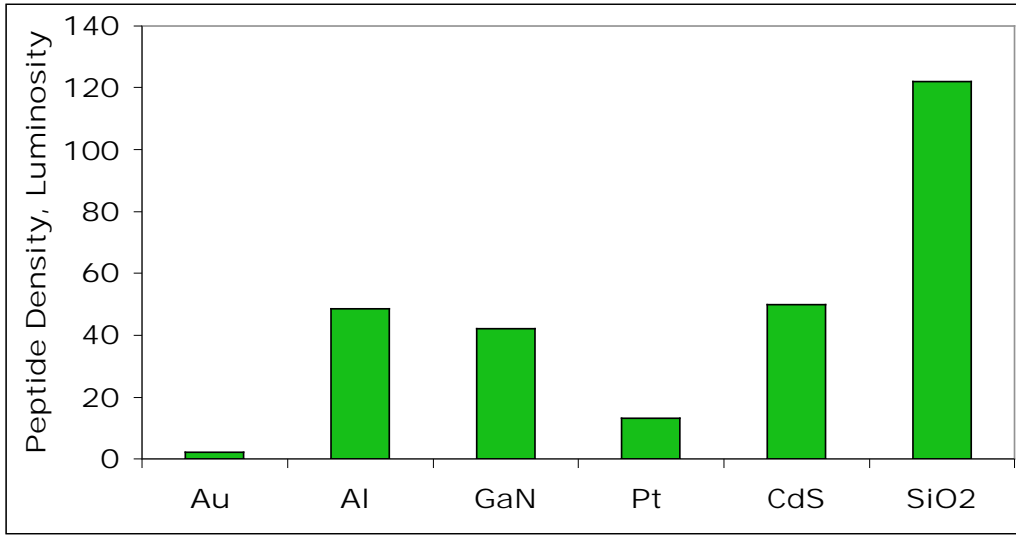


**Figure 2: AO8 Peptide Binding Affinity for Different Materials**

## 2.3 AO8 Binding Affinity

The binding constant for the AO8 peptide for aluminum was derived from the binding half life of peptide on the aluminum surface. The binding constant is equal to the off rate, $k_{off}$ divided by the on rate, $k_{on}$. The on rate is the theoretical diffusion limited on rate, while the o_ rate is simply calculated from the half life, which is obtained experimentally. The measured adhesion half life for the AO8 peptide on aluminum was $1.5 \times 10^5$ s (42 hours).

$$k_d = \frac{k_{off}}{k_{on}} = \frac{Ln(2)/t_{1/2}}{10^8 M^{-1}s^{-1}} = \frac{7 \times 10^{-9} Ms}{1.5 \times 10^5 s} = 5 \times 10^{-14} M$$

The $k_d$ indicates that the AO8 peptide adheres quite strongly to the aluminum electrode. Indeed this is corroborated by rubbing tests; the aluminum substrate with adhered AO8 can be rubbed quite vigorously without the removal of the AO8 peptide.
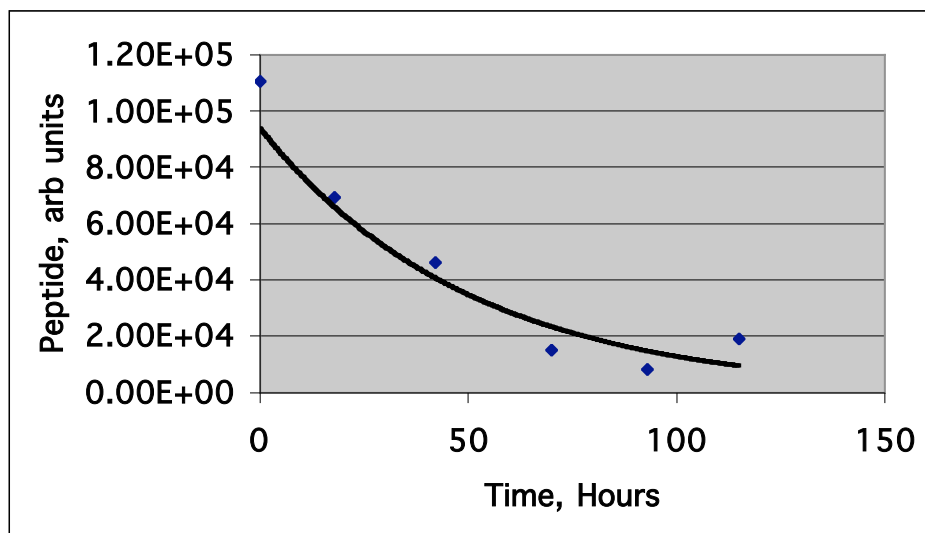
**Figure 3: Determination of AO8 Binding Constant**

## 2.4 AO8 Mutations

A series of modifications to the AO8 peptide were made to better understand the mechanisms controlling the adhesion of this peptide to different materials. For these mutations, either the sequence of the three consecutive arginine groups or three consecutive histidine groups was replaced by a different amino acid.
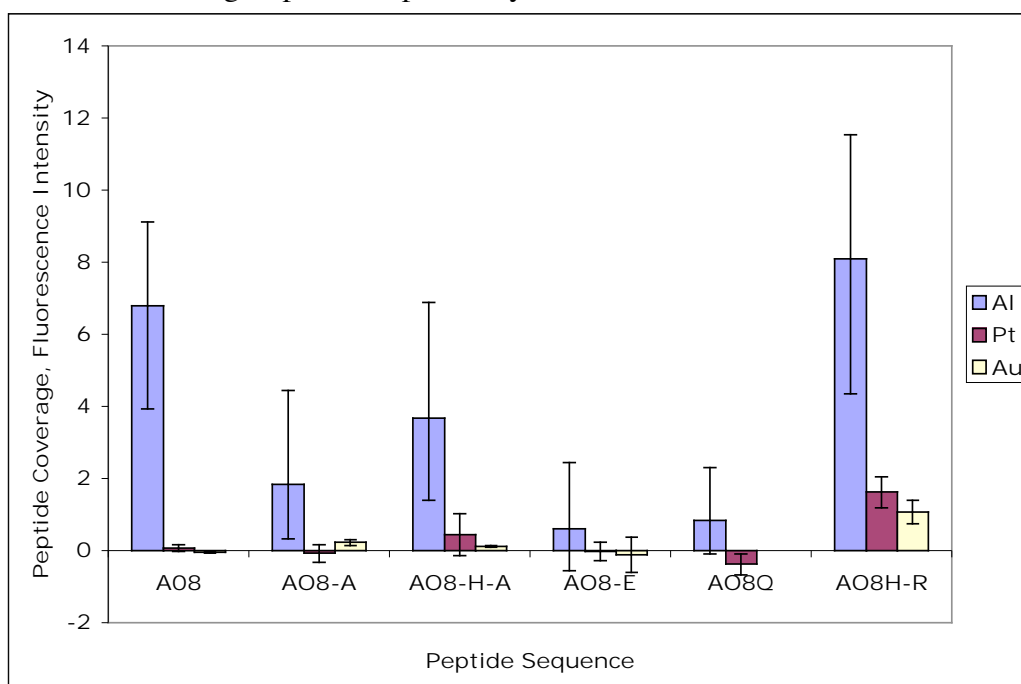


**Figure 4:  Affinity of AO8 mutation peptides to different electrode materials.**

As depicted in Figure 4, the replacement of a either the histidine or arginine with an uncharged or negatively charged group results in reduced binding to aluminum. Replacing histidine with arginine results in some increased binding to aluminum, and

induces binding to gold and platinum.  These results are all indicative of the importance of positive charge in driving peptide binding for these materials.

   In experiments described in the following sections an aluminum electrode is employed, because of both the superior affinity of the AO8 peptide to aluminum and the cost-effectiveness of the material.  Only experiments using the AO8 are described, however the AO8H-R peptide is observed to be have similarly to the AO8 peptide under the influence of an electric field.

**2.5 Reversible Electroresponsive Peptide Using Biorad Electroporation**



Aluminum Electrode
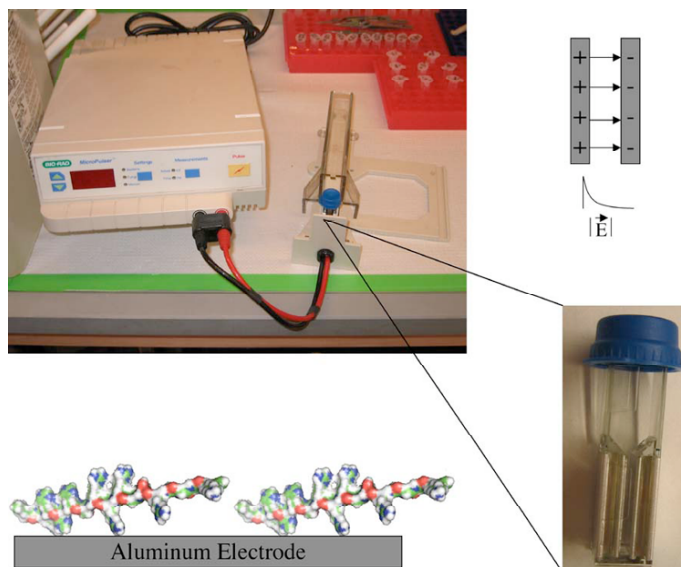
**Figure 5: The Bio-Rad Micropulser Electroporator setup**

A Bio-Rad Micropulser Electroporator produced pulsed electric fields enabling the field driven removal of the AO8 peptide from aluminum electrodes.  This experimental setup is depicted in Figure 5.  The AO8 peptide was attached to the interior aluminum electrodes of an electroporation cuvette (2 mm gap). A measurable transfer of peptide from the positively biased electrode to the negatively biased electrode occurs upon application of twenty 2.5 kV, 5 ms pulses. Increasing the number of pulses or the voltage increases the amount of transfered peptide. This result demonstrates that the binding properties of the A08 peptide are electrically controllable. Fluorescence microscope images of the peptide under varying experimental conditions are shown in Figure 6. The electroporation system is not ideal for this type of experiment because it provides little flexibility; the pulse length and shape cannot be changed, and the electrode geometry is limiting. In the next section the development and testing of a more robust experimental setup is described.
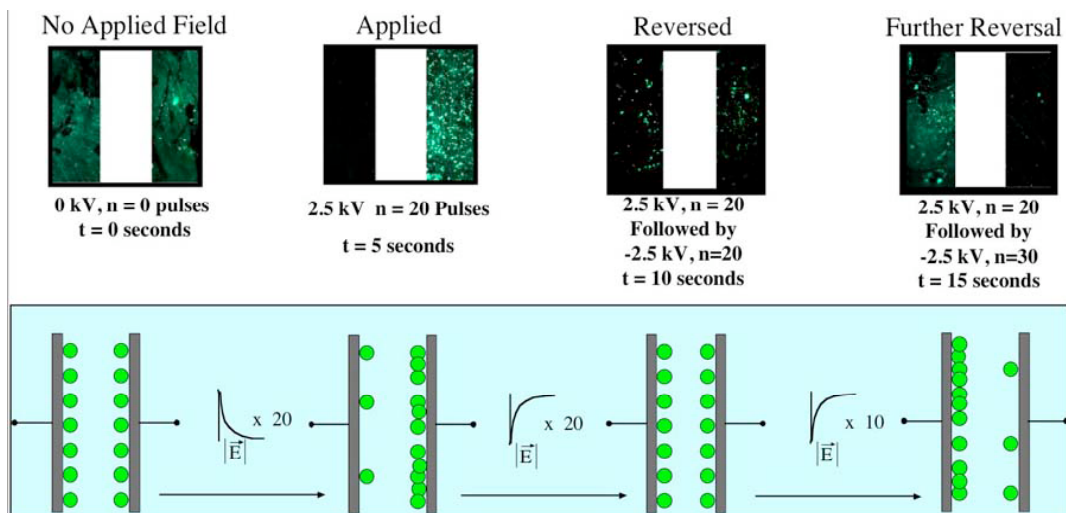
**Figure 6:  A Reversibly Electroresponsive Peptide**

## 2.6 Development of High Voltage Arbitrary Waveform System
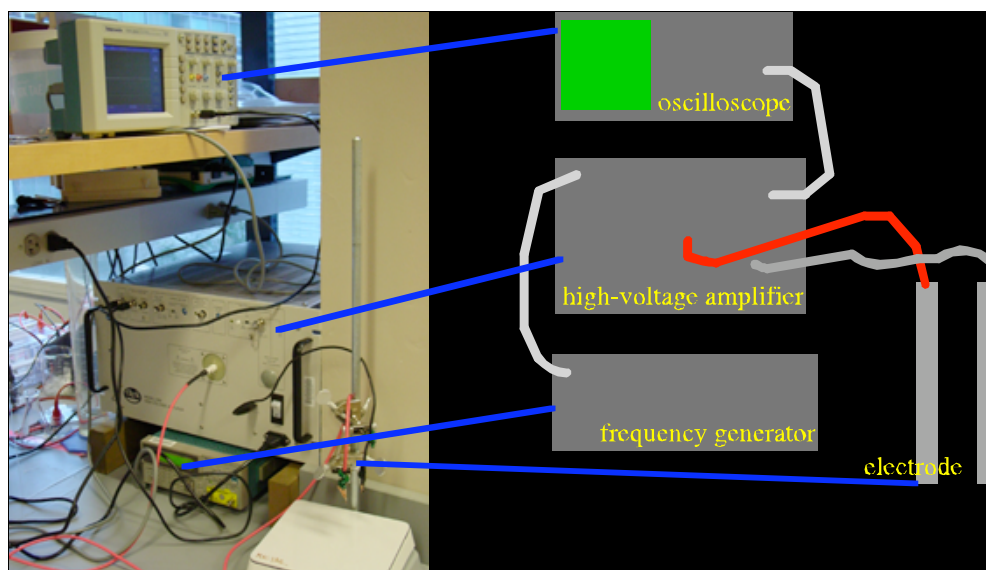


**Figure 7:  Setup for applying high voltage waveforms**

To reversibly apply and remove peptide from an inorganic surface with an electric potential, a pulsed field set-up is necessary. The electroporation apparatus is designed specifically to allow the transformation of e. coli or other organisms. As such it is only able to produce a single exponentially decaying waveform, the decay constant of which is governed by the applied potential. A system which can produce a series of pulses, differing voltages or even a sinusoidal type waveform would be significantly more effective than the Bio-Rad Electropulser.  Figure 7 depicts a set-up that is designed to allow the application of high voltage arbitrary waveforms, enabling the reversible application and removal of peptide from a conductive surface.

With this system it is be possible to probe more precisely the reversible binding behavior of peptides such as A08. Variables that previously were uncontrollable, such as the length of time between pulses, that may lead to discrepancies in data can be precisely controlled

and optimized. Precision in the time between pulses and pulse length enables careful control of the kinetics of peptide transport. Additionally, rather than only having an exponentially decaying waveform, a range of pulse geometries can be studied.

Using the high voltage amplifier system, a more precise characterization of the number of pulses required to transport the peptide between electrodes was conducted. The results of these experiments are shown in Figure 8.  For these experiments a 6.25 kV/mm field with 5 ms/pulse was used.  The results clearly indicate that the peptide is released from one electrode and transported to the opposing electrode.
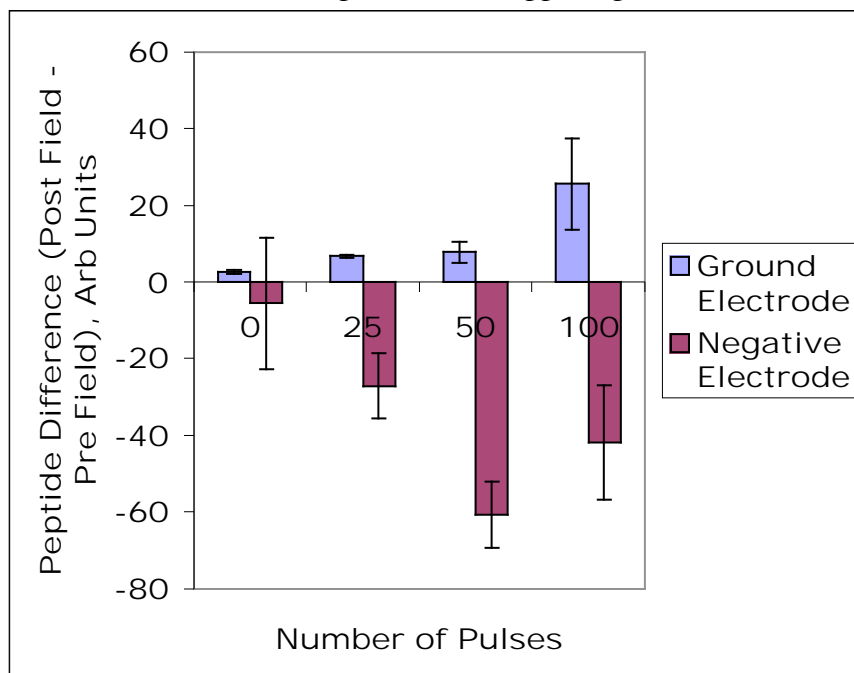


**Figure 8:  The release and transport of the AO8 peptide**

From the number of pulses and the pulse duration the time of transport can be computed.  This can be compared to a theoretically derived number based on electrical drift.  The theoretical drift velocity for a peptide in 6.25 kV/mm field is 43 mm/s.  To travel between electrodes the peptide would take ~93 ms, or about 20 pulses.  Given the rough nature of this estimate, and the possibility of diffusion between pulses, this result is in relatively close agreement to the 50 – 100 pulses it takes to transport the peptide experimentally.
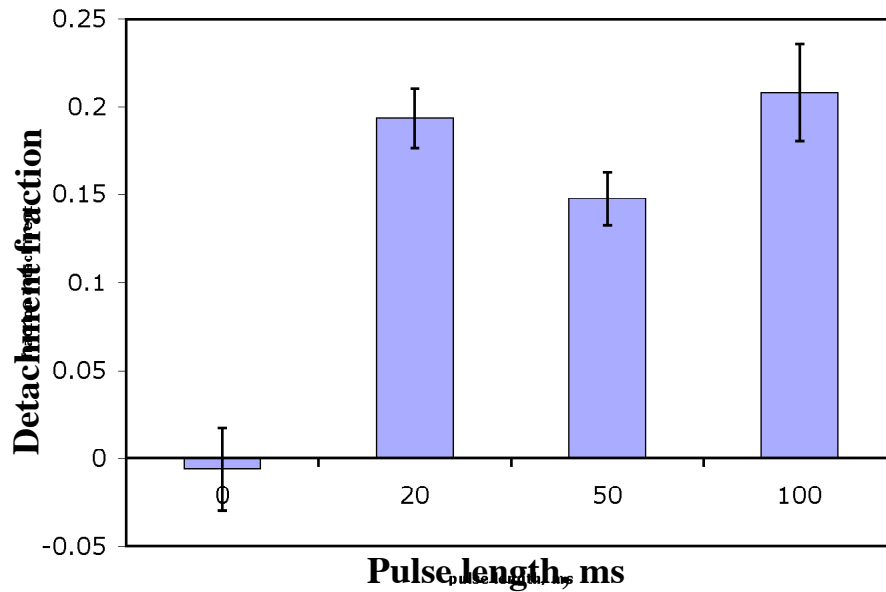
## 2.7 Bipolar Electrode – Removal of Yeast from Sapphire

A surface charge is induced when a semiconductor or insulator is placed in an electric field.  Eric Krauland performed a yeast screen against sapphire, pulling out highly positively charged sequences. The surface of sapphire is highly negatively charged, so it is reasonable to believe electrostatics play an important role in the binding to sapphire. A change in charge of the sapphire surface is therefore expected to counteract the yeast affinity for the surface. The sapphire-binding yeast, expressing the peptide KRHKQKTSRMGK, was bound to the sapphire surface. Using the setup shown in Figure 9, a charge was induced on the sapphire surface. The amount of yeast removed from the surface was determined by comparing images of the sample taken before and after field

exposure. Figure10 shows data for electric field pulses of several different durations.
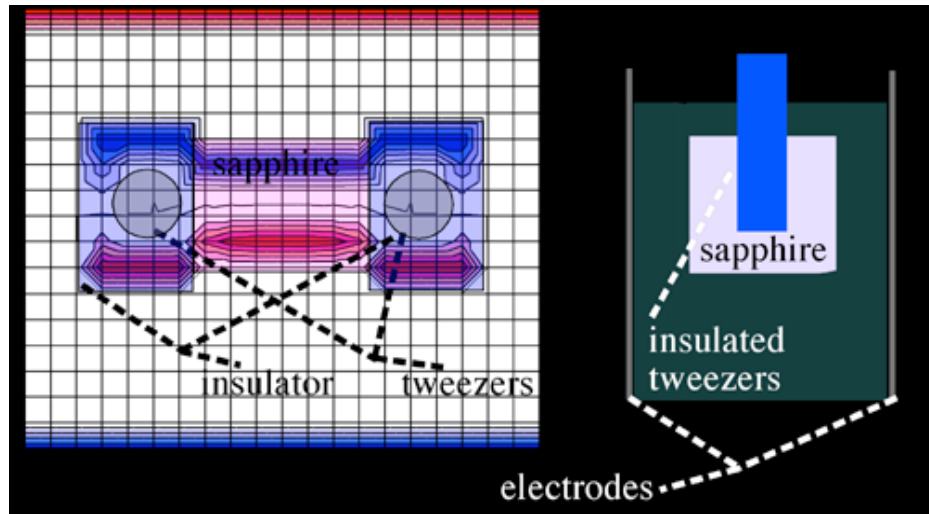


**Figure 9:  Bipolar electrode setup for electrically driven yeast removal.**



**Figure 10:  Field driven removal of yeast from sapphire**

## 2.8 Conclusions

Pulsed electric fields were used to successfully and reversibly release a tightly binding peptide from an electrode surface, and subsequently to transfer the peptide to the opposing electrode where the peptide reattached.  A bipolar electrode configuration was demonstrated enabling the electric field driven release of biomaterials from a nonconductive surface.  Additionally the experiments with yeast on sapphire indicate that

under the right conditions the counteracting peptide and yeast surface charges can be overcome, enabling the electrically stimulated release of yeast from a material surface.

Future experimental work should be focused on three goals. First, by examining the electroresponse of carefully designed peptide sequences, a better understanding of the role individual amino acids play in driving electroresponsive peptide behavior can be developed. Using this information peptide sequences with unique field signature could potentially be developed. The second research focus should be to understand the mechanisms of release and transport of the peptides under the influence of high voltage fields. The transport kinetics in water at these time scales and voltage magnitudes is poorly understood. A firm grasp on the kinetics would enable the engineering of precise microfluidic devices that can controllably deliver peptides or other particles. The final research focus should be to explore the electroresponsive behavior of quantum dots, polymers or other systems where the electroresponsive peptide acts as a linker to the material surface. Electroresponsive quantum dots could be used as highly controllable fluorescent markers, and polymers could be used as an electroresponsive coating.

(6) a. papers in peer reviewed journals:
  • Manuscripts in preparation

  b. Non peer reviewed papers:
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(7) Advanced Degrees: none yet

(8) Inventions: none yet